Journal of Human Nutrition (1980) 34, 251-254.

HEREDOPATHIA ATACTICA POLYNEURITIFORMIS (Refsum's disease)

2. Estimation of phytanic acid in foods

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Estimation of phytanic acid in human plasma and in a number of foodstuffs is described.

The treatment of Refsum's disease by plasma exchange followed by dietary management has been reported recently (Gibberd *et al.*, 1979; Masters-Thomas *et al.*, 1980). After the initial reduction of the plasma phytanic acid of the patient by pla , it was necessary to

were therefore analysed for their phytanic-acid content. This paper describes the methods used for the analysis of plasma and food phytanic acid.

The estimations in plasma are relatively uncomplicated as methyl phytanate C17 acid) position when subjected to gas er cent polyethyleneglycol adipate cid in plasma, the amount of phytanic r, a number of foods contain consider- l interfere with the estimation of L (APL) column the phytanic acid fatty acids (C18), but is well separated foodstuff is hydrogenated, the phytanic acid remains unaffected and the C18 unsaturated acids are converted to stearic acid, the saturated C18 acid, which does not interfere with phytanic acid on the 10 per cent APL column, thus permitting estimation of the latter without error.

Methodology

Preparation of food lipid extracts

Foods that he liked to eat were chosen by the patient and one sample of each purchased by him at his local supermarket was analysed.

Food (100 g) was homogenised with water (300 ml) in a domestic liquidiser. Aliquots of the homogenate were evaporated to dryness in a rotary evaporator under reduced pressure at 70 °C. Aliquots contained approximately 3 g of dry weight of food and their sizes were thus dependent on the water content of the food.

The weighed dried food was extracted with chloroform:methanol (2:1 v/v, 25 ml per g of dried food). The extraction mixture was filtered through fat-free cotton wool and stored at 4 °C.

Preparation of plasma lipid extracts
Plasma (0.5 ml) was extracted with chloroform:methanol (2:1v/v, 6 ml). Methylation was then carried out immediately.

Methylation of lipid extracts
An aliquot of the extraction mixture (3 ml) was hydrolysed with methanolic sodium hydroxide (5 ml, 0.5N) and methylated with boron trifluoride methanol complex (5 ml, 14 per cent), using a modified form of the method of Morrison & Smith (1964). Methyl pentadecanoate (C15) was added as an internal standard (0.1 ml, 0.728 g/l).

The extracted fatty-acid methyl esters were dissolved in n-heptane (0.3 ml) and stored at 4 °C, ready for analysis by GLC on a 10 per cent PEGA column.

Hydrogenation of food GLC sample
The sample prepared for GLC analysis on 10 per cent PEGA was hydrogenated using Adam's platinum catalyst (0.3 g) in methanol (10 ml) and hydrogen at atmospheric pressure.

Upon completion of the hydrogenation, the mixture was refluxed for 30 min. It was then filtered through Hyflo Supercell and the methanol evaporated to dryness under reduced pressure at 50 °C. The residue was redissolved in n-heptane (0.3 ml) and stored at 4 °C, ready for analysis by GLC on 10 per cent APL.

GLC analysis
All GLC analyses were carried out in duplicate using two different types of column: (a) a polar stationary phase, 10 per cent PEGA and (b) a non-polar stationary phase, 10 per cent APL. Analysis of plasma phytanic acid was carried out only on 10 per cent PEGA.

On 10 per cent PEGA, phytanic acid and C17 acid elute together (see Table 1) and cannot be separated for analysis. Since many foodstuffs contain considerable amounts of C17 acid, any sample exhibiting high levels of phytanic acid

Table 1. Log retention volumes of fatty acids relative to methyl pentadecanoate on polar and non-polar phases

Fatty-acid methyl esters	Log retention volume relative to methyl pentadecanoate	
	Polar stationary phase (10% PEGA)	Non-polar stationary phase (10% APL)
Phytanic	0.269	0.439
Heptadecanoic (C17 : C)	0.272	0.355
Stearic (C18 : 0)	0.415	0.527
Oleic (C18 : 1)	0.460	0.465
Linoleic (C18 : 2)	0.545	0.445
Linolenic (C18 : 3)	0.654	0.445

Table 2. Phytanic acid (p.a.) content of foods

Code No. (Paul & Southgate, 1978)	Food	mg p.a. in 100 g dried food	% water	mg p.a. in 100g food
20	White Rice, boiled	3.29	69.9	0.99
28	Spaghetti, canned in tomato sauce (Heinz)	7.00	83.0	1.19
33	White bread	2.70	39.0	1.65
48	Cornflakes	11.20	3.0	1.16
53	'Rice Krispies'	2.83	3.8	2.72
56	'Sugar puffs'	1.09	1.8	1.07
57	'Weetabix'	1.54	3.8	1.48
–	Angel layer cake	12.00	30.0	8.40
–	Custard, made with powder and fresh skimmed milk	3.30	74.7	0.83
157	Cottage cheese	8.62	78.8	1.84
166	Egg white	0.27	88.3	0.03
177	Macaroni cheese, canned (Heinz)	8.00	67.4	2.61
187	Margarine (Flora)	16.75	16.0	14.07
195	Soya oil	14.00	neg.	14.00
259	Beef, stewed, lean	59.00	60.0	23.60
–	Beef, kosher, stewed, lean	28.00	57.1	12.01
–	Veal, stewed	5.00	55.1	2.24
–	Lamb, minced lean and fat, cooked	119.00	59.1	48.67
–	Pork, stewed, lean, meat only	10.00	61.9	3.81
329	Duckling, roast, meat only	22.00	64.2	7.88
351	Rabbit, stewed	6.00	63.9	2.17
394	Ham, boiled, lean	19.38	72.5	5.66
398	Lamb's tongue, canned ('Gold Seal')	6.00	56.2	2.63
–	Liver, pigs, grilled	95.00	62.00	36.10
443	Cod, grilled	19.00	78.0	4.18
456	Smoked haddock, steamed	9.00	71.6	2.56
494	Pilchards, canned in tomato sauce	48.00	70.0	14.46
508	Tuna fish, canned in oil	126.00	54.6	57.21
520	Dressed Crab, canned ('Van Smirren')	24.00	79.2	4.22
569	Baked beans, canned	11.00	73.6	2.90
580	Carrot, fresh, boiled	2.46	90.0	0.25
–	Mushrooms, boiled	4.01	91.5	0.34
631	Chick peas, canned	6.00	65.8	2.05
640	Potato, boiled	3.41	80.5	0.66
650	Potato, instant powder ('Smash')	3.40	7.2	3.15
660	Swede, boiled	1.50	91.6	0.13
–	Textured vegetable protein, dried unflavoured chunks	3.00	neg.	3.00
822	Ground almonds	0.60	4.7	0.57
953	Tomato soup, dried packet ('Knorr')	2.00	4.8	1.90
–	Chicken soup, dried packet ('Knorr')	4.00	2.8	3.89
961	'Marmite'	4.98	25.4	3.73
–	Gravy powder ('Bisto')	1.20	3.0	1.16

(> 10 mg/100 g of dried food) was run
APL column, which separates C17 acid
cent APL, phytanic acid elutes in a bro
Hydrogenation produces a sample cont
separation of phytanic acid is complete.

Calculations

Phytanic acid content of the food (mg/100 g of dry food) =

$$\frac{A}{B} \times \frac{C}{D} \times \frac{Vex}{Vme} \times \frac{17.6}{\text{Wt of dry food}}$$

where: A = Peak area (PA) of phytanic acid in food
B = PA of C15 acid in food
C = PA of C15 acid in mixed standard
D = PA of phytanic acid in mixed standard
Vex = Volume of extraction solvent
Vme = Volume of extraction solvent used for methylation

Mixed standard = 0.1 methyl pentadecanoate (0.728 g/l) and 0.2 ml methyl phytanate (0.88 g/l). Phytanic acid content of plasma (mg/100ml)=

$$\frac{E}{F} \times \frac{C}{D} \times 76.267$$

where: E = PA of phytanic acid in plasma
F = PA of C15 acid in plasma.

Results

The phytanic content of a range of foods has been analysed with the aim of permitting an intake of solid food up to the level of 10 mg phytanic acid per day. Foods may contain differing amounts of phytanic acid due to seasonal variations and geological conditions and these factors require further study. The method of calculating the amount of phytanic acid in the food is shown below and the phytanic acid contents are shown in Table 2.

Wt of phytanic acid in untreated food =

$$\frac{W}{100} \times W\left(1 - \frac{Y}{100}\right)$$

where: W = mg phytanic acid per 100 g of dried food
Y = % water in food.

Acknowledgement—we are indebted to the endowment funds of Westminster Hospital for generous financial assistance.

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